EXHIBIT C

22 A method of detecting the presence of a carbohydrate antigen characteristic of at least one species or [serogruop] serogroup of a species of bacteria in a fluid, which method comprises the following steps:

[(a) culturing an identified species, or serogroup of a species of bacteria , to a desired size and harvesting therefrom cells of that species, or serogroup of a species of bacteria, as a wet cell pellet;]

[(b)] (a) obtaining from [the wet cell pellet] a culture of a known species, or serogroup of a species of bacteria an essentially protein-free carbohydrate antigen [by a series of substeps which comprise

(i). suspending the wet cell pellet from step (a) in an alkaline solution and mixing;

(ii). adjusting the pH to an acid pH with a strong acid;

(iii). separating the mixture from substep (b) (ii) into two layers;

(iv). removing the upper layer and adjusting its pH to approximate neutrality;

(v). adding to the product from substep (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi). adjusting the pH of the product from substep (b) (v) to an alkaline pH with a weakly alkaline aqueous solution and;

(vii). separating out an essentially protein free carbohydrate antigen of said]

These pads are especially chosen to hold the dry conjugate and to release it when wetted by sample.

The nitrocellulose pad is first treated by individually embedded affinity purified *anti-Haemophilus influenza* b antibodies into a first portion of the pad. These antibodies act as the capture lines. A control line is established by striping goat anti-rabbit IgG on the surface of the pad. For those lines which are striped on the nitrocellulose pad, a solution consisting of 5mM sodium phosphate, pH 7.4 containing 5 per cent methanol and 0.102 percent Intrawhite dye is used as a carrier fluid for the antibodies. The nitrocellulose pad is then desiccated at a temperature of 18-25 ^{o} C. to promote permanent protein absorption thereto.

The absorbent pad used is of cellulosic material sold in commerce as Ahlstrom 243. It requires no special treatment. All the pads are assembled in the order shown in Figure 1C on the adhesive strip when the test devise is put together for delivery to the customer.

C. Immunoassay Procedure

In the conduct of the assay according to the invention, finished test devices having the swab well, the overlayers with holes and test strips arranged as shown in the Figures are utilized. A swab fashioned from fibrous Dacron is briefly immersed in the urine sample and is then removed from the sample and immediately inserted, through the overlayer hole B on the right-hand side of the device, into the sample well of the test device. Two or three drops of "Reagent A", in this case a solution of 2.0 percent Tween 20, 0.05 percent sodium azide and 0.5 percent sodiumm dodecyl sulfate in a 0.05 M sodium citrate-sodium phosphate buffer of pH 6.5 are added to the sample through the same hole. The adhesive strip on the edge of the right-hand side is peeled away and the device is then closed. The sample immediately contacts

the second paragraph of this section. The fact that raw polyvalent antibodies to bacterial carbohydrate antigens may be rendered highly antigen-specific and sensitive by subjecting them to affinity purification with a purified target bacterial carbohydrate antigen that is essentially protein-free likewise has not been appreciated heretofore. Likewise, the fact that carbohydrate antigens from both Gram-negative and Gram-positive bacteria and/or from the capsular layer surrounding both types of bacteria can all be purified and used to affinity purify antibodies to such antigens to yield antigen-specific antibodies has not been heretofore recognized, nor has it been appreciated that bacterial carbohydrate antigens can be detected rapidly with high accuracy, sensitivity and specifity using such antigen-specific antibodies as a detecting agent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 and its related Figures 1A, 1B and 1C depict a typical device of the type preferred in the performance of an assay for a bacterial carbohydrate antigen in accordance with this invention.

Figures 2, 3 and 4 are graphs showing, in Figure 2, the ability of antigen-specific purified antibodies of this invention to detect other serotypes of *H. influenza* type b than the one to which the antibodies were raised. In Figures 3 and 4, the graphs reflect that the purified antigen-specific antibodies of *H. influenza* type b were not cross-reactive with antigens of H. influenza types a, c, d or f (Fig.3) or with any of nontypical H. influenza NT1, NT2, NT3 or NT4 or with H. *para-influenza.*

DETAILED DESCRIPTION OF THE INVENTION

The present invention represents an exceptional advance in methods for detecting bacteria infection.

Because it is applicable to the detection in mammalian bodily fluids of bacterial carbohydrate antigens of all known types–i.e., the lipo-polycarbohydrate antigens including lipo-polysaccharides, the antigen lip-teichoic acids and their antigenic derivatives and the capsular polycarbohydrate antigens, including polysaccharides– and it represents a unified approach to the detection of bacterial infection not heretofore envisioned, this invention holds promise for permitting the rapid diagnoses of virtually any bacteria-caused disease wherein the bacteria possess a carbohydrate antigen that manifest itself in the disease state in a bodily fliud of the patient

Of particular importance is the opportunity that this invention affords for rapid diagnosis and rapid introduction of appropriate therapy in situations where a particular bacterially-caused disease appears to be epidemic within a group–whether a small, confined group, e.g., in a school or geriatric center, or a widespread population as, e.g., a town, a city or a larger region.

Broadly speaking, the preferred immunochromatographic ("ICT") assay of this invention may be designed and configures to be run on any known disposable ICT device disclosed in the art. Preferably it is designed to be conducted, and is conducted, using an ICT device of the type disclosed in co-pending U.S. Patent Application Serial No. 07/706,639 of Howard Candler, now U.S. Patent 6,168,956, or one of its continuation-in-part applications, all of which are assigned to Smith-Kline Diagnostics, Inc. but are exclusively licensed ti Binax, Inc. (which is entitled to assign-